growth factor-alpha in human keratinocytes. *Nature* 328:817–20

Cook PW, Pittelkow MR, Shipley GD (1991) Growth factor-independent proliferation of normal human neonatal keratinocytes: production of autocrine- and paracrine-acting mitogenic factors. *J Cell Physiol* 146:277–89

Cook PW, Piepkorn M, Clegg CH *et al*. (1997) Transgenic expression of the human amphiregulin gene induces a psoriasis-like phenotype. *J Clin Invest* 100:2286–94

Hashimoto K, Higashiyama S, Asada H *et al*. (1994) Heparin-binding epidermal growth factor-like growth factor is an autocrine growth factor for human keratinocytes. *J Biol Chem* 269:20060–6

Kansra S, Stoll SW, Johnson JL *et al*. (2004) Autocrine extracellular signal-regulated kinase (ERK) activation in normal human keratinocytes: metalloproteinase-mediated release of amphiregulin triggers signaling from ErbB1 to ERK. *Mol Biol Cell* 15:4299–309

Kansra S, Stoll SW, Johnson JL *et al*. (2005) Src family kinase inhibitors block amphiregulin-mediated autocrine ErbB signaling in normal human keratinocytes. *Mol Pharmacol* 67:1145-57

Lu X, Wang Q, Hu G *et al*. (2009) ADAMTS1 and MMP1 proteolytically engage EGF-like ligands in an osteolytic signaling cascade for bone metastasis. *Genes Dev* 23:1882–94

Shirakata Y, Komurasaki T, Toyoda H *et al*. Epiregulin (2000), a novel member of the epidermal growth factor family, is an autocrine growth factor in normal human keratinocytes. *J Biol Chem* 275:5748–53

Sternlicht MD, Sunnarborg SW, Kouros-Mehr H *et al*. (2005) Mammary ductal morphogenesis requires paracrine activation of stromal EGFR via ADAM17-dependent shedding of epithelial amphiregulin. *Development* 132:3923–33

Stoll SW, Johnson JL, Li Y *et al*. (2010) Amphiregulin carboxy-terminal domain is required for autocrine keratinocyte growth. *J Invest Dermatol* 130:2031–40

Strachan L, Murison JG, Prestidge RL *et al*. (2001) Cloning and biological activity of epigen, a novel member of the epidermal growth factor superfamily. *J Biol Chem* 276:18265–71

See related article on pg 2017

Protein Kinase C/Mitogen-Activated Protein Kinase Signaling in Keratinocyte Differentiation Control

Mitchell F. Denning[1]

Proper epidermal keratinocyte differentiation, which is necessary for cutaneous barrier function, is altered in many common skin diseases. Keratinocyte differentiation is controlled by a complex signaling network involving multiple members of the protein kinase C and mitogen-activated protein kinase signaling kinases. Using an RNA interference knockdown approach, Adhikary *et al.* identified essential nodes in this signaling network, revealing remarkable kinase specificity.

Journal of Investigative Dermatology (2010) **130**, 1968–1970. doi:10.1038/jid.2010.194

Most of us take differentiation of our epidermis for granted. Our skin reliably provides adequate barrier function and the aesthetic appearance associated with a normal, healthy skin. For a significant number of people, however, epidermal differentiation does not proceed normally, and their skin is a chronic source of irritation, inflammation, and aggravation. Several of the most common skin diseases, including eczema (atopic dermatitis), ichthyosis (vulgaris, lamellar, and X-linked), and psoriasis, are associated with fundamental defects in epidermal differentiation. These defects are not always simply a response to an underlying disease processes; often they are pathogenic. For example, ichthyosis vulgaris and atopic dermatitis are linked to loss-of-function mutations in the intermediate filament bundling protein filaggrin (Smith *et al*., 2006; Palmer *et al*., 2006). Because disruption of epidermal differentiation is responsible for a considerable portion of the morbidity associated with these diseases, a better understanding of the basic processes that control keratinocyte differentiation is needed to achieve pharmacological improvement for the tens of millions of people who suffer from them.

Adhikary *et al*. (2010, this issue) focus on the transcriptional regulation of involucrin, a constituent of the cornified envelope and resident of the epidermal differentiation complex on chromosome 1q21. Involucrin is expressed in the spinous and granular layers of the epidermis and is transcriptionally upregulated by a variety of differentiating agents, e.g., calcium, 12-O-tetradecanoylphorbol-13-acetate (TPA), vitamin D, and (−)-epigallocatechin-3-gallate (EGCG). Although involucrin-null mice appear normal and have no obvious skin phenotype (Djian *et al*., 2000), involucrin induction is part of the coordinated transcriptional response during keratinocyte differentiation, and it can be considered a prototypical keratinocyte differentiation marker that shares regulatory overlap with many genes residing in the epidermal differentiation complex. Thus, involucrin is a reasonable surrogate for keratinocyte differentiation. In addition, several of the critical signaling kinases identified as important for involucrin induction are also important for morphological changes that accompany differentiation.

The findings of Adhikary *et al*. (2010) help delineate a signaling network involving protein kinase C (PKC)-δ, MEKK-1, MEK-3, MEK-6, and p38-δ as essential to activate activating protein 1 nuclear translocation, DNA binding, and involucrin gene expression by multiple differentiation stimuli (Figure 1). PKC-η is also implicated in involucrin induction by TPA, but not Ca^{2+} or EGCG. PKC isoforms can activate mitogen-activated protein kinase (MAPK) pathways via multiple mechanisms, and overexpression of both PKC-δ and

[1]*Department of Pathology and the Cardinal Bernardin Cancer Center, Skin Cancer Research Program, Loyola University Chicago, Maywood, Illinois, USA*

Correspondence: Mitchell F. Denning, Department of Pathology and the Cardinal Bernardin Cancer Center, Skin Cancer Research Program, Loyola University Chicago, 2160 South First Avenue, Maywood, Illinois 60153, USA. E-mail: mdennin@lumc.edu

Clinical Implications

- Many of the most common skin diseases involve alterations in keratinocyte differentiation.
- Defective keratinocyte differentiation is not only a symptom but also an instigator of some dermatoses.
- Identification of key signaling networks regulating epidermal differentiation may permit pharmacological rescue of normal keratinocyte differentiation.

PKC-η has previously been shown to activate p38-δ (Efimova *et al*., 2002). It is noteworthy that only specific members of the PKC and MAPK pathways are required for involucrin induction, and the issue of signaling specificity is highlighted in this Commentary.

PKC specificity

Activation of the PKC family of serine/threonine kinases, by multiple agonists, is a potent inducer of keratinocyte differentiation. Although at least five PKC isoforms (α, δ, ε, η, and ζ) are expressed in the epidermis, Adhikary *et al*. (2010) identified PKC-δ as having the primary role in KC differentiation induced by TPA, calcium, and EGCG. PKC-δ is a member of the Ca^{2+}-independent "novel" subclass of PKC isoforms, and it is highly expressed in both mouse and human epidermis. PKC-δ translocates to the membrane in response to extracellular calcium, most likely in response to diacylglycerol generated by phospholipase C (Denning *et al*., 1995). However, no specific alterations in subcellular localization have been noted in differentiating suprabasal keratinocytes *in vivo* (D'Costa *et al*., 2006). In addition, there are no major defects in the epidermis of PKC-δ-null mice; thus, the role of PKC-δ in normal epidermal differentiation requires more study. PKC-δ has also been strongly linked to apoptosis in keratinocytes and other cell types in response to genotoxic agents, such as UV radiation (Denning *et al*., 1998). Although similarities exist between the programmed cell death pathways of keratinocyte terminal differentiation and apoptosis (destruction of organelles, genetically programmed), many notable differences are also evident.

It is uncertain what specifies PKC-δ activation leading to a differentiated or apoptotic cell fate, but it may be related to the magnitude, duration, and location of PKC-δ activation. Activation of PKC-δ by differentiating agents is modest and transient, whereas PKC-δ cleavage and activation by genotoxic apoptotic agents is substantial and sustained. PKC-δ activation mechanisms also differ, with differentiating stimuli inducing a transient activation of the full-length PKC-δ via agonist (diacylglycerol, TPA) binding to the C1 domain. In contrast, apoptotic stimuli induce proteolytic cleavage of PKC-δ, which separates the regulatory domain from the catalytic domain to generate a constitutively active catalytic fragment (Denning *et al*., 1998). Compared with classical agonist-induced activation, this cleavage activation mechanism results in altered PKC-δ subcellular distribution and possibly substrate access. PKC scaffold proteins, such as the receptors for activated C kinase (RACKs), are involved in determining PKC isoforms' functional selectivity by restricting substrate access (Churchill *et al*., 2009). RACKs interact with PKC isoforms via their C2 domains, and these vary in sequence among PKC isoforms. Thus, unlike the proteolytic catalytic fragment of PKC-δ, which lacks its C2 domain, the full-length PKC-δ activated by diacylglycerol or TPA should be bound to its RACK. Furthermore, PKC isoform-selective RACK binding may also explain why PKC-δ has a role in involucrin induction whereas the closely related PKC-ε does not.

PKC-η is also implicated by Adhikary *et al*. (2010) in the induction of involucrin promoter activation by TPA. PKC-η is unique among PKC isoforms in that its expression is restricted largely to cells undergoing squamous differentiation (Koizumi *et al*., 1993). The finding

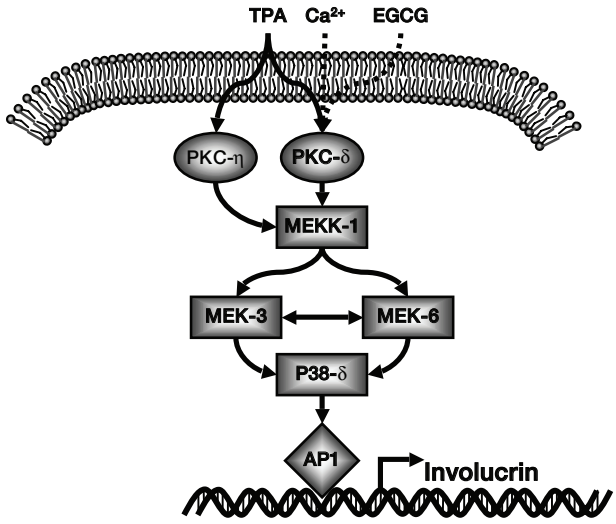

Figure 1. Hypothetical signaling pathway leading to involucrin gene expression. The induction of involucrin promoter activation by the PKC agonist TPA involves both PKC-η and PKC-δ, whereas induction of involucrin by extracellular Ca^{2+} or EGCG requires PKC-δ predominantly. The activation of PKC-δ by Ca^{2+} and EGCG is indirect, as indicated by dotted lines. MEKK-1, MEK-3, MEK-6 and p38-δ are each involved in induction of involucrin by TPA. Both MEK-3 and MEK-6 are required for involucrin promoter activation, suggesting that they may lie in the same pathway or activate each other. These signaling kinases are also required for nuclear localization and DNA-binding activity of activating protein 1 (AP1) transcription factors (JunB, JunD, c-fos, and Fra-1). EGCG, (−)-epigallocatechin-3-gallate; PKC, protein kinase C; TPA, 12-O-tetradecanoylphorbol-13-acetate.

that PKC-η is not required for involucrin promoter activation by Ca^{2+} or EGCG supports the idea that individual differentiation-inducing agents exhibit specific mechanisms of action. The requirement for PKC-η in TPA-induced involucrin expression is consistent with previous findings from the Eckert lab showing that overexpression of PKC-η can induce involucrin (Efimova *et al*., 2002). However, these earlier studies found that PKC-ε overexpression also induced involucrin, whereas the current study clearly shows that PKC-ε is not involved in involucrin induction by TPA. These conflicting results highlight the need for caution when interpreting results from overexpression studies and reinforce the importance of loss-of-function approaches, such as RNA interference.

MAPK specificity

Although p38-α, -β, and -δ are all expressed in keratinocytes, only siRNA knockdown of p38-δ inhibits involucrin induction. This selectivity for p38-δ raises the question of how specificity is conferred on individual components of the MAPK signaling pathway. MAPK's signaling modules—consisting of MAPK, MAPK kinases, and MAPK kinase kinases—are often coordinately activated by binding to specific scaffolding or adapter proteins. In the case of p38 MAPKs, scaffolding proteins such as JIP2 and JLP have been identified, but it is unclear whether they are specific for individual p38 isoforms. Earlier work by the Eckert lab identified p38-δ as the only p38 isoform activated by several inducers of differentiation (TPA, calcium, okadaic acid), and the current RNA interference results are consistent with that observation (Efimova *et al*., 2002). Thus, although it is clear that p38-δ is the p38 isoform responsible for involucrin induction, the mechanism of this selectivity remains elusive. Earlier studies on involucrin gene expression, also by the Eckert lab, found an inverse regulation between p38-α and p38-δ involving MEK-6, and this type of cross-inhibition may be another explanation for p38 isoform functional selectivity (Dashti *et al*., 2001).

The p38 MAPKs can be activated by MEK-3/MKK-3 and MEK-6/MKK-6, and knockdown of each of these kinases significantly reduced involucrin promoter activation. This lack of redundancy suggests that MEK-3 and MEK-6 are dependent on each other and thus may lie in the same pathway or stimulate the activation of each other. Additional studies looking at activation of MEK-3 in cells lacking MEK-6, and vice versa, are needed to resolve this finding.

Pharmacological rescue of differentiation

The impact of defective epidermal keratinocyte differentiation on human health is enormous. For many patients with dermatoses involving abnormal epidermal differentiation, symptomatic relief comes from inhibiting the inflammatory response to altered epidermal barrier function with topical steroids and relieving dryness with moisturizing creams and oils. Pharmacological approaches to restoring normal skin homeostasis directly in these patients should be more effective and well tolerated. Given the numerous examples in knockout mice of compensation among epidermal differentiation structural proteins, a more thorough understanding of signaling networks that regulate genes within the epidermal differentiation complex, such as involucrin, may reveal targets for drugs capable of restoring proper epidermal keratinocyte differentiation.

CONFLICT OF INTEREST

The author states no conflict of interest.

ACKNOWLEDGMENTS

The author thanks Leonid Sitailo and Edward LaGory for reading this Commentary and providing insightful comments.

REFERENCES

Adhikary G, Chew YC, Reece EA *et al.* (2010) PKC-δ and -η, MEKK-1, MEK-6, MEK-3, and p38-δ are essential mediators of the response of normal human epidermal keratinocytes to differentiating agents. *J Invest Dermatol* 130:2017–30

Churchill EN, Qvit N, Mochly-Rosen D (2009) Rationally designed peptide regulators of protein kinase C. *Trends Endocrinol Metab* 20:25–33

Dashti SR, Efimova T, Eckert RL (2001) MEK6 regulates human involucrin gene expression via a p38α- and p38δ-dependent mechanism. *J Biol Chem* 276:27214–20

D'Costa AM, Robinson JK, Maududi T *et al.* (2006) The proapoptotic tumor suppressor protein kinase C-δ is lost in human squamous cell carcinomas. *Oncogene* 25:378–86

Denning MF, Dlugosz AA, Williams EK *et al.* (1995). Specific protein kinase C isozymes mediate the induction of keratinocyte differentiation markers by calcium. *Cell Growth Differ* 6:149–57

Denning MF, Wang Y, Nickoloff BJ *et al.* (1998) Protein kinase Cδ is activated by caspase-dependent proteolysis during ultraviolet radiation-induced apoptosis of human keratinocytes. *J Biol Chem* 273:29995–30002

Djian P, Easley K, Green H (2000) Targeted ablation of the murine involucrin gene. *J Cell Biol* 151:381–8

Efimova T, Deucher A, Kuroki T *et al.* (2002) Novel protein kinase C isoforms regulate human keratinocyte differentiation by activating a p38δ mitogen-activated protein kinase cascade that targets CCAAT/enhancer-binding protein α. *J Biol Chem* 277: 31753–60

Koizumi H, Kohno Y, Osada S *et al.* (1993) Differentiation-associated localization of nPKC η, a Ca^{2+}-independent protein kinase C, in normal human skin and skin diseases. *J Invest Dermatol* 101:858–63

Palmer CN, Irvine AD, Terron-Kwiatkowski A *et al.* (2006) Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 38:441–6

Smith FJ, Irvine AD, Terron-Kwiatkowski A *et al.* (2006) Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat Genet* 38:337–42